

## I. AMENDMENTS TO THE CLAIMS

Claim 1 (Withdrawn): A crystallized *M. tuberculosis* isocitrate lyase enzyme (ICL) characterized as:

- (a) a crystallized apo-ICL that has the crystallographic data of Table 1;
- (b) a crystallized apo-ICL that has the crystallographic coordinates of FIG. 5, as deposited in the Protein Data Bank under accession code 1F61;
- (c) a crystallized ICL in complex with the inhibitor 3-bromopyruvate and having the crystallographic coordinates of FIG. 6, as deposited in the Protein Data Bank under accession code 1F8M; or
- (d) a crystallized ICL in complex with the inhibitor 3-nitropropionate and having the crystallographic coordinates of FIG. 7, as deposited in the Protein Data Bank under accession code 1F8I.

Claim 2 (Withdrawn): The crystallized ICL of claim 1, wherein said crystallized ICL is a crystallized apo-ICL that has the crystallographic data of Table 1.

Claim 3 (Withdrawn): The crystallized ICL of claim 1, wherein said crystallized ICL is a crystallized apo-ICL that has the crystallographic coordinates of FIG. 5, as deposited in the Protein Data Bank under accession code 1F61.

Claim 4 (Withdrawn): The crystallized ICL of claim 1, wherein said crystallized ICL is a crystallized ICL in complex with the inhibitor 3-bromopyruvate and having the crystallographic coordinates of FIG. 6, as deposited in the Protein Data Bank under accession code 1F8M.

Claim 5 (Withdrawn): The crystallized ICL of claim 1, wherein said crystallized ICL is a crystallized ICL in complex with the inhibitor 3-nitropropionate and having the crystallographic coordinates of FIG. 7, as deposited in the Protein Data Bank under accession code 1F8I.

**Claim 6 (Withdrawn):** A computer-readable data storage medium comprising a data storage material encoded with computer-readable data, wherein said data comprises:

- (a) the structure coordinates of apo-ICL according to FIG. 5, as deposited in the Protein Data Bank under accession code 1F61;
- (b) the structure coordinates of the ICL-3-brompyruvate complex according to FIG. 6, as deposited in the Protein Data Bank under accession code 1F8M;
- (c) the structure coordinates of the ICL-3-nitropropionate complex according to FIG. 7, as deposited in the Protein Data Bank under accession code 1F8I; or
- (d) the structure coordinates of ICL amino acids His 193, Asn 313, Ser 315 and Ser 317 according to FIG. 5, FIG. 6 or FIG. 7.

**Claim 7 (Withdrawn):** The data storage medium of claim 6, wherein said computer-readable data comprises:

- (a) the structure coordinates of apo-ICL according to FIG. 5, as deposited in the Protein Data Bank under accession code 1F61;
- (b) the structure coordinates of the ICL-3-brompyruvate complex according to FIG. 6, as deposited in the Protein Data Bank under accession code 1F8M; or
- (c) the structure coordinates of the ICL-3-nitropropionate complex according to FIG. 7, as deposited in the Protein Data Bank under accession code 1F8I.

**Claim 8 (Withdrawn):** The data storage medium of claim 6, wherein said computer-readable data comprises the structure coordinates of ICL amino acids His 193, Asn 313, Ser 315 and Ser 317 according to FIG. 5, FIG. 6 or FIG. 7.

**Claim 9 (Withdrawn):** A computer-readable data storage medium comprising a data storage material encoded with computer-readable data, wherein said data comprises the structure coordinates of ICL amino acids His 193, Asn 313, Ser 315 and Ser 317 according to FIG. 5, FIG.

6 or FIG. 7; wherein amino acids His 193, Asn 313, Ser 315 and Ser 317 mediate closure of the active site loop upon binding of an inhibitor to ICL.

Claim 10 (Withdrawn): A computer for producing a three-dimensional representation of:

- (a) a molecule or molecular complex comprising the structure coordinates of FIG. 5 (Protein Data Bank accession code 1F61), FIG. 6 (Protein Data Bank accession code 1F8M) or FIG. 7 (Protein Data Bank accession code 1F8I); or
- (b) a homologue of said molecule or molecular complex, wherein said homologue comprises structure coordinates that have a root mean square deviation from the backbone atoms of the amino acids of the structure coordinates of FIG. 5, FIG. 6 or FIG. 7 of not more than 1.5 angstroms; wherein said computer comprises:
  - (i) a computer-readable data storage medium comprising a data storage material encoded with computer-readable data, wherein said data comprises the structure coordinates of FIG. 5, FIG. 6 or FIG. 7;
  - (ii) a working memory for storing instructions for processing said computer-readable data;
  - (iii) a central-processing unit coupled to said working memory and to said computer-readable data storage medium for processing said computer-machine readable data into said three-dimensional representation; and
  - (iv) a display coupled to said central-processing unit for displaying said three-dimensional representation.

Claim 11 (Withdrawn): A computer for producing a three-dimensional representation of:

- (a) a molecule or molecular complex that comprises an active site loop defined by the structure coordinates of ICL amino acids His 193, Asn 313, Ser 315 and Ser 317 according to FIG. 5, FIG. 6 or FIG. 7; wherein amino acids His 193, Asn 313, Ser

315 and Ser 317 mediate closure of the active site loop upon binding of an inhibitor to ICL; or

- (b) a homologue of said molecule or molecular complex, wherein said homologue comprises an active site loop that has a root mean square deviation from the backbone atoms of said amino acids of not more than 1.5 angstroms, wherein said computer comprises:
  - (i) a computer-readable data storage medium comprising a data storage material encoded with computer-readable data, wherein said data comprises the structure coordinates of ICL amino acids His 193, Asn 313, Ser 315 and Ser 317 according to FIG. 5, FIG. 6 or FIG. 7;
  - (ii) a working memory for storing instructions for processing said computer-readable data;
  - (iii) a central-processing unit coupled to said working memory and to said computer-readable data storage medium for processing said computer-machine readable data into said three-dimensional representation; and
  - (iv) a display coupled to said central-processing unit for displaying said three-dimensional representation.

Claim 12 (Withdrawn): A computer for determining at least a portion of the structure coordinates corresponding to X-ray diffraction data obtained from a molecule or molecular complex, wherein said computer comprises:

- (a) a computer-readable data storage medium comprising a data storage material encoded with machine-readable data, wherein said data comprises at least a portion of the structural coordinates of apo-ICL according to FIG. 5 (Protein Data Bank accession code 1F61), ICL-3-brompyruvate complex according to FIG. 6 (Protein Data Bank accession code 1F8M) or ICL-3-nitropropionate complex according to FIG. 7 (Protein Data Bank accession code 1F8I);

- (b) a computer-readable data storage medium comprising a data storage material encoded with computer-readable data, wherein said data comprises X-ray diffraction data obtained from said molecule or molecular complex;
- (c) a working memory for storing instructions for processing said computer-readable data of (a) and (b);
- (d) a central-processing unit coupled to said working memory and to said computer-readable data storage medium of (a) and (b) for performing a Fourier transform of the machine readable data of (a) and for processing said computer-readable data of (b) into structure coordinates; and
- (e) a display coupled to said central-processing unit for displaying said structure coordinates of said molecule or molecular complex.

Claim 13 (Withdrawn): The computer of claim 12, wherein said computer comprises a computer-readable data storage medium comprising a data storage material encoded with machine-readable data, wherein said data comprises the structural coordinates of apo-ICL according to FIG. 5 (1F61), ICL-3-brompyruvate complex according to FIG. 6 (1F8M) or ICL-3-nitropropionate complex according to FIG. 7 (1F8I).

Claim 14 (Withdrawn): The computer of claim 12, wherein said molecule or molecular complex comprises a polypeptide having isocitrate lyase enzyme activity.

Claim 15 (Withdrawn): A method for evaluating the potential of a candidate compound to associate with:

- (a) a molecule or molecular complex that comprises an active site loop defined by the structure coordinates of ICL amino acids His 193, Asn 313, Ser 315 and Ser 317 according to FIG. 5, FIG. 6 or FIG. 7; or
- (b) a homologue of said molecule or molecular complex, wherein said homologue comprises an active site loop that has a root mean square deviation from the

backbone atoms of said amino acids of not more than 1.5 angstroms; wherein said method comprises the steps of:

- (i) employing computational means to perform a fitting operation between said candidate compound and an active site loop defined by the structure coordinates of ICL amino acids His 193, Asn 313, Ser 315 and Ser 317 according to FIG. 5, FIG. 6 or FIG. 7 within a root mean square deviation from the backbone atoms of said amino acids of not more than 1.5 angstroms; and
- (ii) analyzing the results of said fitting operation to quantify the association between said candidate compound and said active site loop.

Claim 16 (Withdrawn): The method of claim 15, wherein said method evaluates the potential of said candidate compound to associate with:

- (a) a molecule or molecular complex defined by structure coordinates of all amino acids of ICL, wherein said structure coordinates are selected from the group consisting of apo-ICL according to FIG. 5 (1F61), ICL-3-bromopyruvate complex according to FIG. 6 (1F8M) and ICL-3-nitropropionate complex according to FIG. 7 (1F8I); or
- (b) a homologue of said molecule or molecular complex having a root mean square deviation from the backbone atoms of said amino acids of not more than 1.5 angstroms.

Claim 17 (Amended): A method for identifying an inhibitor of a microbial isocitrate lyase enzyme (ICL), comprising:

- (a) defining the catalytic active site of ICL from the atomic coordinates selected from the group consisting of the atomic coordinates for the apo-ICL of FIG. 5 (Protein Data Bank accession code 1F61), the atomic coordinates for the ICL-3-

bromopyruvate complex of FIG. 6 (Protein Data Bank accession code 1F8M) and the atomic coordinates for the ICL-3-nitropropionate complex of FIG. 7 (Protein Data Bank accession code 1F8I); and

- (b) identifying a ~~non-native~~ substrate compound that fits said active site and thereby inhibits said microbial isocitrate lyase enzyme.

Claim 18 (Amended): A method for identifying an inhibitor of a microbial ICL, comprising:

- (a) obtaining atomic coordinates of ICL, wherein said atomic coordinates are selected from the group consisting of the apo-ICL atomic coordinates of FIG. 5 (Protein Data Bank accession code 1F61), the ICL-3-bromopyruvate complex atomic coordinates of FIG. 6 (Protein Data Bank accession code 1F8M) and the ICL-3-nitropropionate complex atomic coordinates of FIG. 7 (Protein Data Bank accession code 1F8I);
- (b) defining the catalytic active site of ICL from said atomic coordinates; and
- (c) identifying a ~~non-native~~ substrate compound that fits said catalytic active site, wherein a non-native substrate compound that fits said active site is indicative of an inhibitor of a microbial ICL.

Claim 19 (Amended): The method of claim 18, wherein said ~~non-native~~ substrate compound that fits said catalytic active site is identified by selecting a candidate compound and confirming that said candidate compound inhibits said microbial ICL.

Claim 20 (Amended): A method for identifying an inhibitor of a microbial ICL, comprising:

- (a) obtaining atomic coordinates of ICL, wherein said atomic coordinates are selected from the group consisting of the apo-ICL atomic coordinates of FIG. 5 (Protein Data Bank accession code 1F61), the ICL-3-bromopyruvate complex atomic coordinates of FIG. 6 (Protein Data Bank accession code 1F8M) and the ICL-3-

nitropropionate complex atomic coordinates of FIG. 7 (Protein Data Bank accession code 1F8I);

- (b) defining the catalytic active site of ICL from said atomic coordinates;
- (c) selecting a candidate compound by identifying a ~~non-native~~ substrate compound that fits said catalytic active site; and
- (d) contacting said microbial ICL with said candidate compound under conditions effective for ICL activity, wherein a candidate compound that inhibits the activity of said microbial ICL is confirmed as an inhibitor of said microbial ICL.

Claim 21 (Original): The method of claim 20, wherein said candidate compound is selected from consideration of a database of compounds.

Claim 22 (Original): The method of claim 20, wherein said candidate compound is selected by *de novo* design.

Claim 23 (Original): The method of claim 20, wherein said candidate compound is selected by design starting from a known inhibitor.

Claim 24 (Original): The method of claim 20, wherein said candidate compound is selected by identifying a compound intended to interact with at least one of ICL amino acids His 193, Asn 313, Ser 315 or Ser 317 according to FIG. 5, FIG. 6 or FIG. 7.

Claim 25 (Original): The method of claim 24, wherein a candidate compound intended to interact with at least one of said ICL amino acids His 193, Asn 313, Ser 315 or Ser 317 is selected by employing computational means to perform a fitting operation between said candidate compound and a binding pocket defined by said amino acids within a root mean square deviation from the backbone atoms of said amino acids of not more than 1.5 angstroms.

Claim 26 (Original): The method of claim 20, wherein the inhibitor so identified inhibits a mycobacterial ICL.



Claim 27 (Original): The method of claim 26, wherein the inhibitor so identified inhibits *M. tuberculosis* ICL.

Claim 28 (Original): The method of claim 20, wherein the inhibitor so identified inhibits a fungal ICL.

Claim 29 (Original): The method of claim 20, wherein the inhibitor so identified inhibits said microbial ICL by changing the structure of said active site from the open conformation to the closed conformation.

Claim 30 (Original): The method of claim 20, wherein the inhibitor so identified is a competitive inhibitor.

Claim 31 (Original): The method of claim 20, wherein the inhibitor so identified is a non-competitive or uncompetitive inhibitor.

Claim 32 (Original): The method of claim 20, further comprising purifying or synthesizing the inhibitor so identified.

Claim 33 (Original): The method of claim 20, further comprising formulating the inhibitor so identified in a pharmaceutically acceptable formulation.

Claim 34 (Original): The method of claim 33, wherein said pharmaceutically acceptable formulation further comprises at least a second antimicrobial agent.

Claim 35 (Withdrawn): A method for identifying a potential agonist or antagonist of a molecule comprising a microbial ICL-like active site loop, said method comprising the steps of:

- (a) using the atomic coordinates of ICL amino acids His 193, Asn 313, Ser 315 and Ser 317 according to FIG. 5, FIG. 6 or FIG. 7 within a root mean square deviation from the backbone atoms of said amino acids of not more than 1.5 angstroms, to generate a three-dimensional structure of molecule comprising a microbial ICL-like active site loop;

- (b) employing said three-dimensional structure to design or select said potential agonist or antagonist;
- (c) purifying or synthesizing said potential agonist or antagonist; and
- (d) contacting said potential agonist or antagonist with said molecule to determine the ability of said potential agonist or antagonist to interact with said molecule.

Claim 36 (Withdrawn): The method of claim 35, wherein step (a) comprises using the atomic coordinates of all the amino acids of ICL according to FIG. 5, FIG. 6 or FIG. 7 within a root mean square deviation from the backbone atoms of said amino acids of not more than 1.5 angstroms.

Claim 37 (Withdrawn): A method for identifying an agent for use in the treatment of chronic tuberculosis, comprising:

- (a) obtaining atomic coordinates of *M. tuberculosis* ICL, wherein said atomic coordinates are selected from the group consisting of the apo-ICL atomic coordinates of FIG. 5 (Protein Data Bank accession code 1F61), the ICL-3-bromopyruvate complex atomic coordinates of FIG. 6 (Protein Data Bank accession code 1F8M) and the ICL-3-nitropropionate complex atomic coordinates of FIG. 7 (Protein Data Bank accession code 1F8I);
- (b) defining the catalytic active site of ICL from said atomic coordinates;
- (c) selecting a candidate compound by identifying a non-native substrate compound that fits said catalytic active site; and
- (d) contacting *M. tuberculosis* ICL with said candidate compound under conditions effective for ICL activity, wherein a candidate compound that inhibits the activity of said *M. tuberculosis* ICL is indicative of an agent for use in the treatment of chronic tuberculosis.

Claim 38 (Withdrawn): A method for identifying an agent for use in treating or preventing a persistent microbial infection, comprising selecting an enzyme target from the glyoxylate shunt pathway in an intracellular microbial pathogen and identifying a compound that inhibits said enzyme target, thereby identifying an agent for use in treating or preventing a persistent microbial infection.

Claim 39 (Withdrawn): The method of claim 38, wherein said selected enzyme target is isocitrate lyase or malate synthase.

Claim 40 (Withdrawn): The method of claim 38, wherein said selected enzyme target is an enzyme from the glyoxylate shunt pathway in a mycobacterium.

Claim 41 (Withdrawn): The method of claim 38, wherein said selected enzyme target is an enzyme from the glyoxylate shunt pathway in a fungus.

Claim 42 (Withdrawn): The method of claim 38, wherein a compound that inhibits the selected enzyme target is identified by testing the ability of said compound to inhibit the growth of said intracellular microbial pathogen when grown on a carbon source *in vitro* that mimics the nutrient environment encountered during the persistent phase of infection *in vivo*.

Claim 43 (Withdrawn): The method of claim 42, wherein a compound that inhibits the selected enzyme target is identified by testing the ability of said compound to differentially inhibit the growth of said intracellular microbial pathogen when grown *in vitro* on a C<sub>2</sub> carbon source versus a C<sub>6</sub> carbon source.

Claim 44 (Withdrawn): The method of claim 38, wherein a compound that inhibits the selected enzyme target is identified by a method comprising:

- (a) testing the ability of said compound to inhibit the activity of the selected enzyme target in a cell-free enzyme activity assay; and
- (b) confirming the ability of said compound to significantly inhibit the growth of said intracellular microbial pathogen when grown on acetate *in vitro*, but to not

significantly inhibit the growth of said intracellular microbial pathogen when grown on glucose *in vitro*, thereby favoring the selection of an inhibitor that preferentially inhibits an enzyme of the glyoxylate shunt pathway operative in persistent infection of inflammatory macrophages *in vivo*.

Claim 45 (Withdrawn): The method of claim 38, wherein a compound that inhibits the selected enzyme target is identified by selecting a candidate inhibitor based upon the crystal structure of said selected enzyme target.